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Abbreviated Title: **Direct LSC of Wood-Sorbed ^{14}C Organics**

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Abstract

Wet ashing, substrate decolorization, and a solvent extraction approach were all tested in an attempt to quantify the retention of [^{14}C]2,4-dichlorophenol and [^{14}C]2,4,5-trichlorophenol by pulped wood fibers. Development of a method for determining the mass of fiber contained in counting vials as part of the solvent extraction approach allowed experiments to be carried out using small quantities of fiber. The combination of solvent extraction and this mass determination method produced recoveries of > 98%, proving it to be the superior approach.

Introduction

The partitioning of an aqueous organic compound between solution and solid phases is often quantified by a distribution coefficient, K_d, which is the equilibrium ratio between the solid (C_s) and water (C_w) phase concentrations. Oftentimes, the most effective approach for measuring the distribution of an organic chemical is through the use of ^{14}C labeled radioisotopes, which enable quantitative measurements at extremely low concentrations (Slater, 1990). For compounds that have a strong affinity to the solid phase, experimental measurements of K_d are easily made from changes in solution phase ^{14}C activity. But for compounds that sorb less strongly, the measurements of distribution coefficients are complicated by the need to make a direct assay of the activity retained by the sorbent.

The sorption of ^{14}C labeled chlorophenols to pulped wood fibers is an example where the activity of the solid-sorbed solute must be measured (Severtson, 1993). The direct liquid scintillation

counting of isotopes sorbed to the fibers is interfered with by color quenching and self-absorption. Color quenching is caused by residual amounts of the darkly colored lignin material which composes between 0-15% of the mass of a processed fiber depending on the grade of paper being produced. Figure 1 demonstrates the effect of increasing lignin levels on the counting efficiency of [^{14}C]2,4,5-trichlorophenol. In this case, the radioactivity was added after the scintillant to isolate color quenching effects by avoiding isotope sorption. Both curves extrapolate back to the same initial efficiency levels at zero mass, but while the fibers with their color completely bleached out have no effect on the scintillation counting, increasing the amount of fibers containing lignin (11%) dramatically reduces counting efficiencies. This heavy color quenching combined with potential self-absorption losses make it impossible to accurately measure sorbed ^{14}C activity by simply counting the fibers.

Conventional means for eliminating error caused by color quenching and self-absorption involve the dissolution and decolorization of the adsorbent (Slater, 1990; Neame, 1974), but the combined presence of lignin and cellulose makes wood materials difficult to dissolve without strong thermal and chemical treatment. This can result in large isotope losses especially in the case of volatile organics such as chlorophenols. This study compares three simple recovery techniques for quantifying the sorption of ^{14}C labeled organics to wood fibers without the need for extensive processing. The first involves the wet ashing of fibers and the sampling of $^{14}CO_2$ from an alkaline trap (Spiller, 1993). This is the most complicated of the three approaches because it involves the chemical treatment of the fibers outside the scintillation vial. In the other two techniques, the fibers are treated directly in the scintillation vials prior to counting. In one approach, the color of the fibers is removed using domestic

bleach, and the compounds are presumed to be desorbed from the solid phase by the addition of scintillation cocktail (Smith, 1987). For the other, the fibers are leached using methanol and distilled water to desorb the isotope, and efficiency corrections are made from quench curves produced from dried fibers.

Experimental Procedures

Materials

Pulp fibers obtained from Georgia-Pacific Corporation were thoroughly washed to remove residual processing chemicals, and the average lignin content of each sample was determined (TAPPI Press 1994). The fibers were centrifuged to a 30% consistency (solids content) and stored at 5°C. The consistency of the samples was never allowed to rise above 35% to preserve the pore structure of the fibers which collapses with drying. Radiolabeled chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Both [^{14}C]2,4-dichlorophenol (8.42 mCi/mmol) and [^{14}C]2,4,5-trichlorophenol (4.40 mCi/mmol) were stored in amber vials as methanol solutions at 2°C. 2,4-Dichlorophenol was > 98% pure, and 2,4,5-trichlorophenol was > 99% pure as determined by HPLC. Scintillation counting was done on a Beckman (Fullerton, CA) Model LS 3801 Scintillation Counter. Scintiverse E Scintillation Cocktail (Fisher, Pittsburgh, PA) was used for all solution phase samples and for solid samples from the decolorization technique. Sigma-Fluor™ Universal LSC Cocktail was used with the fiber samples from the methanol/water extraction approach.

Recovery Experiments

For the adsorption reactions in the wet ashing and substrate decolorization procedure, 8 mL of pH 7 buffered, distilled water was combined with 100 mg (oven dried) pulp fibers in amber vials with Teflon-lined caps. A high-fiber concentration was used in all recovery experiments to enhance the retention by the fiber phase. Either [^{14}C]2,4-dichlorophenol (57.6 nCi) or [^{14}C]2,4,5-trichlorophenol (18.42 nCi) was added through a methanol carrier, and the components were thoroughly mixed and allowed to equilibrate. No change was detected in the sorption of isotopes after the first day of equilibration, and a four-day equilibration period was chosen. The fibers were then separated from the aqueous phase using vacuum filtration and placed either in a 12-mL sealable glass vial for wet ashing or directly into a 20-mL scintillation vial for decolorization. For mass balance determinations, triplicate measurements were made of the separated solution phase at a window setting of 300-670, and efficiency corrections were carried out with chemical quench curves generated using ^{14}C standards purchased from Beckman.

The wet ashing procedure was based on a method described by Spiller and Stallings (1993). Initially, the fibers are decomposed with 3 mL of concentrated H_2SO_4 which produces a dark brown solution. A 30% H_2O_2 solution (300 µL) is then added, and the mixture is gently hand mixed to further facilitate oxidation. After about 10 minutes, the resulting yellow solution is heated clear on a hotplate at 85°C for 10 minutes. The entire procedure is carried out in a sealed vial containing a 1.5-mL conical microcentrifuge tube filled with 500 µL of 2-N NaOH solution. The NaOH solution acts as a trap for the $^{14}CO_2$ released from chlorophenol compounds which are oxidized along with fibers. After the

mixture cools, volume adjustments are done on the trap solution, and 100-μL aliquots are counted to determine the total activity contained in the fibers. Counting was done at a window setting of 300-670, and efficiencies were determined with the chemical quench curves.

The substrate decolorization technique is a modified version of the method described by Smith and Lang (1987). Here, the fibers were placed directly into a loosely capped 20-mL glass scintillation vial and decolorized using 3 mL of a 25% (v/v) domestic bleach solution (equivalent to 1.25% sodium hypochlorite) at 55°C for 2 hours. The decolorized sample was then treated with 45 μL of 4-M NH_4OH solution to remove the strong chemiluminescence caused by the unreacted hypochlorite. After a lag of 30 minutes to allow the nitrogen bubbles from the reaction between the ammonia and hypochlorite to dissipate, 100 μL of acetic acid and 17 mL of scintillation cocktail were added, and the samples were incubated for 12 hours. Counting was done at a window setting of 400-670 to avoid the added background counts from the chemiluminescence of bleached samples. Quench corrections were performed using chemical quench efficiency curves generated for this window setting.

For the solvent extraction procedure, water (4 mL) was combined with 50 mg of pulp fibers and either 28.8 nCi of [^{14}C]2,4-dichlorophenol or 9.21 nCi of [^{14}C]2,4,5-trichlorophenol fibers in amber vials with Teflon-lined caps. After four days, the fibers were separated from the aqueous phase using vacuum filtration and placed in 20-mL scintillation vials. Methanol (4.32 mL), distilled water (4.32 mL), and 180 μL of glacial acetic acid (to reduce chemiluminescence) were added; the vials were mixed and incubated for 15 minutes; and 12 mL of scintillant was then added. H number was

determined daily, and it was found that five days of clarification was sufficient for the samples to reach an equilibrium H number (i.e., a minimum). The samples were then counted with a window setting of 0-670. Color quench curves were generated by adding various amounts of pulp fibers ranging from 40 to 60 mg. With the quenching from the brown color of the fibers being the only factor of concern in the generation of the quench curves, the fibers could be dried to much higher consistencies allowing for significantly more accurate mass measurements.

Results and Discussion

The purpose of testing three recovery techniques was the failure of the first two to provide an accurate method for measuring isotope distributions. Table 1 lists the fiber-phase [^{14}C]2,4-dichlorophenol and [^{14}C]2,4,5-trichlorophenol recoveries for the various techniques. Each value is an average of seven measurements. Initially, the wet ashing procedure appeared to be the most promising approach because it avoided liquid scintillation counting of the sorbent. But the recoveries were highly variable and frequently fell below 50%. The decolorization of fibers was successful in eliminating color quenching, but the thermal treatment and nitrogen released from the ammonia reaction with residual hypochlorite appeared to strip the compound out of solution. This resulted in recoveries of < 70%. Since these techniques failed to eliminate the interference caused by the fibers in counting the sorbed isotopes, the remaining alternative was to correct for these interferences with external standards.

The use of color quench curves (Fig. 2) generated from dried fiber in combination with the methanol/water extraction approach proved to be superior in accounting for the sorbed chlorophenols.

All recoveries were > 98%, and most were > 99% with excellent reproducibility (< ± 1.5). The mechanism assumed in this approach is the generation of identical chemical quenching for each sample, and the nearly complete desorption of isotopes from the fiber. Chemical quenching variations were kept to a minimum by repeating the identical procedure including the use of the same equipment and brand of supplies for all samples. This allows the use of a single correction for the combined chemical and color quenching losses. The physical nature of the bond typically involved in the sorption of neutral organics to purely organic substrates (Wu, 1986; Weber, 1991) should allow for the complete desorption of the retained organics with the correct solvent combination. The choice of methanol and water for leaching the fiber samples was based on the need to both disperse the fibers (water) and modify the hydrophobic forces (methanol). This combination produced an excellent dispersion of the fibers even after 12 mL of the scintillation cocktail were added, allowing for an even settling of the fibers to produce highly reproducible equilibrium H number measurements.

Although the extraction procedure was highly successful in recovering sorbed isotopes, there were major difficulties in applying it to sorption measurements. Mass levels of the samples had to be limited to around 50 mg to optimize the accuracy of the quench corrections and could only be used within about a 20-mg range (e.g., 40 to 60 mg). Since pulped wood fibers are mainly composed of cellulose, they tend to retain moisture which adds considerable uncertainty to fiber weight. With the need to maintain the pore structure of the fibers for the sorption experiments, the 50-mg fiber samples were weighed at a consistency of about 35% (65% moisture). This results in mass errors of as much as

20%, and more than 20% error in the measured distribution coefficient. Thus, the benefits of a highly accurate recovery technique do not translate into accurate distribution coefficients.

Fortunately, the even settling of fibers in the scintillation vials produced an H number measure which was highly sensitive to mass variations. As with color quenching during liquid scintillation counting, the photons released by the scintillant during external standardization (induced by the external ^{137}Cs gamma source or "pea") are absorbed by the lignin in the pulp fibers. This light absorption (as measured by H number) was found to be proportional to the concentration of the absorbing substance (as measured by fiber mass and lignin content), similar to Beer's Law. This is demonstrated in Figure 3 which is a plot of mass (oven dried) versus H number for fibers containing 11% lignin. The line is simply a plot of the data used in Figure 2, but instead of efficiency, the oven-dried mass that produced the quenching is plotted. This allows the mass of fiber in the scintillation vial to be measured using H number. The application of the solvent extraction approach in combination with quench functions and this mass determination technique is demonstrated in Figure 4 for the sorption isotherm of [^{14}C] labeled 2,4,5-trichlorophenol by pulped wood fibers. The plot shows the data from the initial fit in which the mass for the solid phase concentration measures is based on attempts to accurately weigh 50 mg of fiber (on oven-dried basis). It also shows the significantly better fit (an r^2 value of 0.995 vs. 0.924) produced from data in which the "mass determination function" is used for the same calculation. This accuracy is crucial in following the effect of variables such as pH and inorganic salt concentrations on the retention of organic materials by fibrous solids.

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Table 1. Percent recoveries of ^{14}C labeled chlorophenols from pulped wood fibers.

lignin mass fraction in fiber	*0.036*	*0.11*	*0.16*
wet ashing			
2,4-dichlorophenol	48.4	68.7	56.9
2,4,5-trichlorophenol	61.3	43.6	49.3
substrate decolorization			
2,4-dichlorophenol	61.7	67.8	64.4
2,4,5-trichlorophenol	70.7	62.4	63.8
solvent extraction			
2,4-dichlorophenol	98.4	99.2	98.7
2,4,5-trichlorophenol	99.4	99.1	100

Captions to Figures

Fig. 1. The effects of fiber mass on the liquid scintillation counting efficiency of [^{14}C]2,4,5-trichlorophenol for fibers at two lignin levels.

Fig. 2. ^{14}C quench curve for pulped fibers of 11% lignin content ($r^2 = 0.97$).

Fig. 3. Mass correction for fibers of 11% lignin content ($r^2 = 0.96$).

Fig. 4. Sorption isotherm linear fits with ($C_s = 0.00980 + 41.6C_w$, $r^2 = 0.995$) and without ($C_s = 0.0152 + 39.3C_w$, $r^2 = 0.924$) the use of the mass determination technique for the retention of 2,4,5-trichlorophenol to pulped fibers of 11% lignin content.

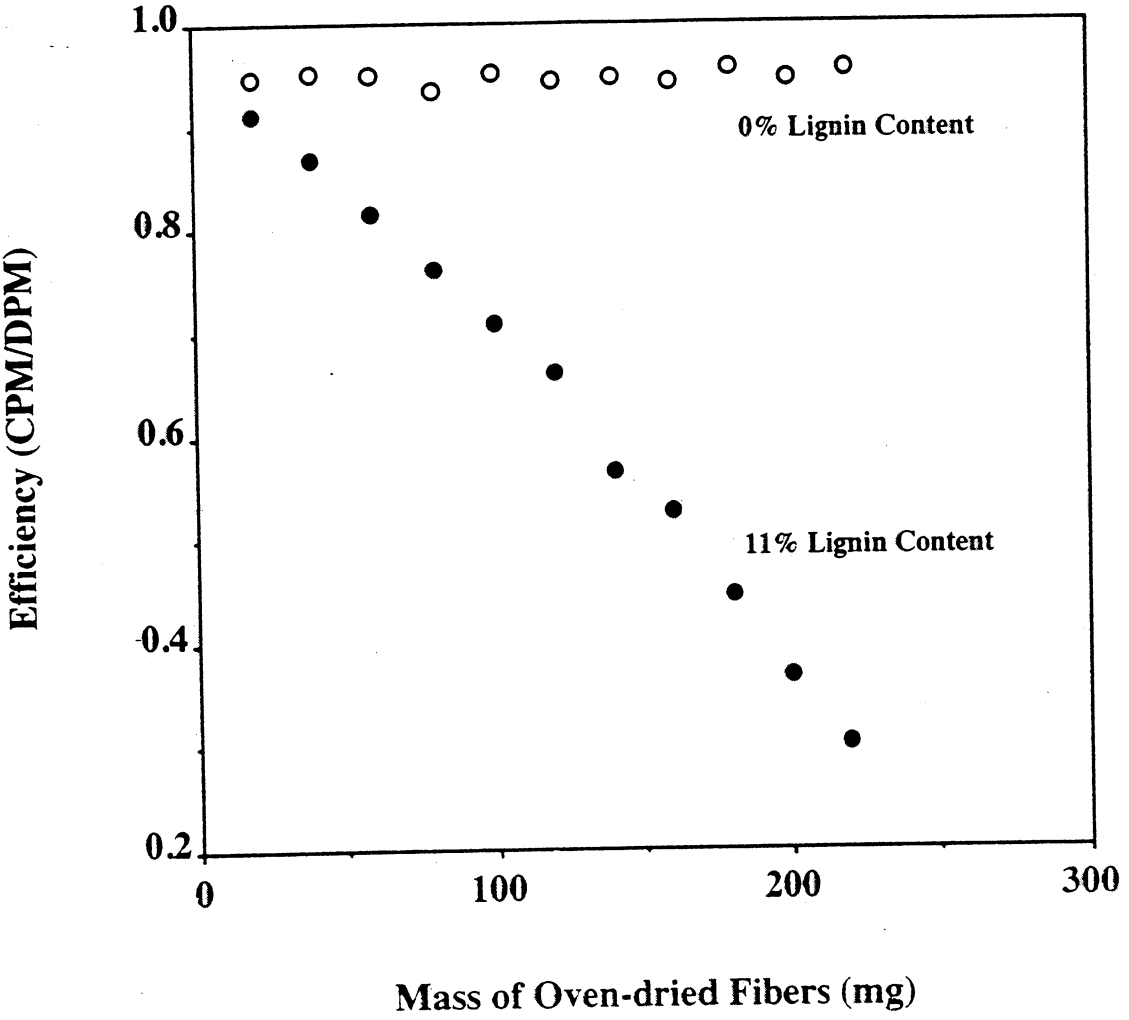
1.0
0.8
0.6
0.4
0.2
0
100
200
300
Efficiency (CPM/DPM)
Mass of Oven-dried Fibers (mg)
0% Lignin Content
11% Lignin Content

Fig. 1

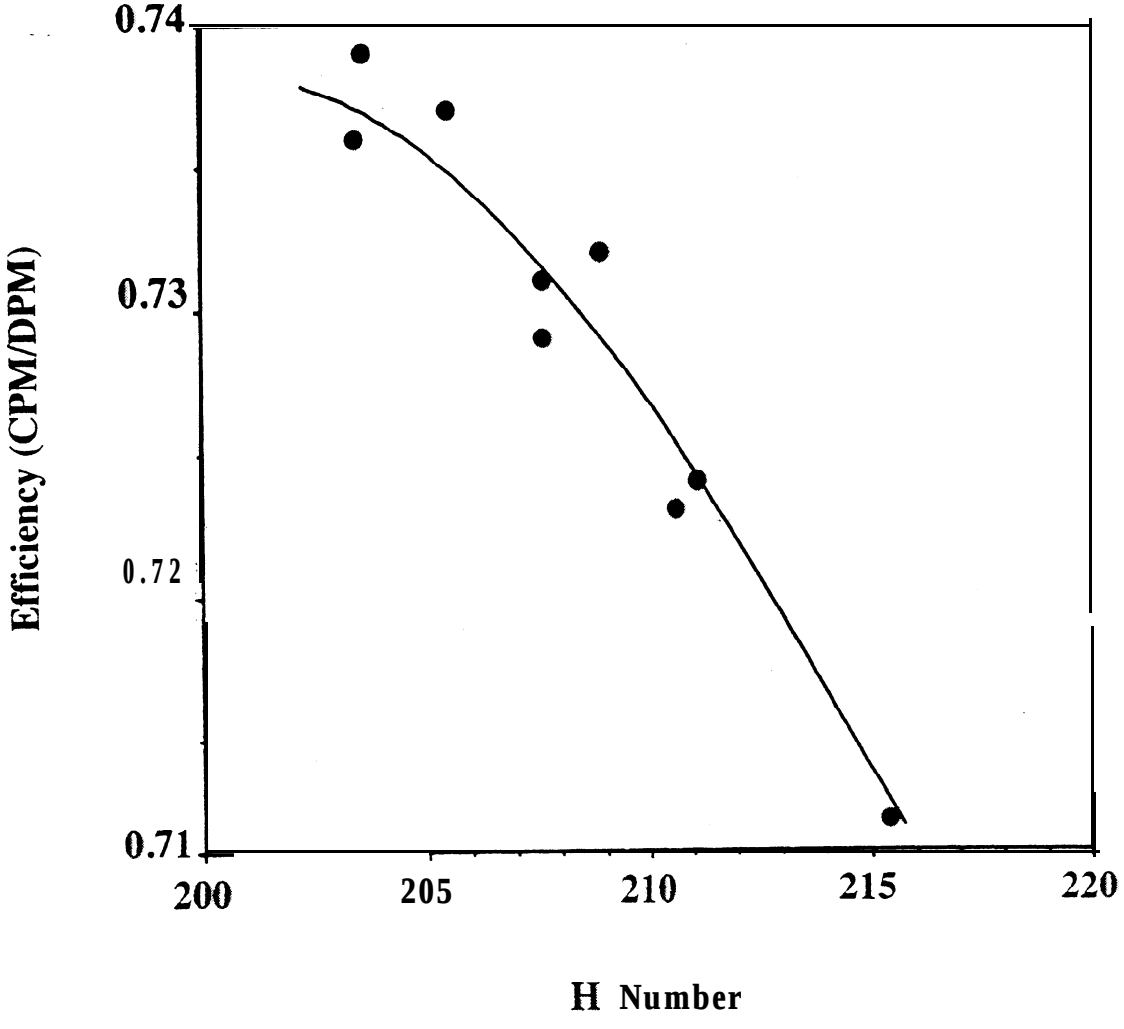
0.74
0.73
0.72
0.71
Efficiency (CPM/DPM)
200
205
210
215
220
H Number

Fig. 2

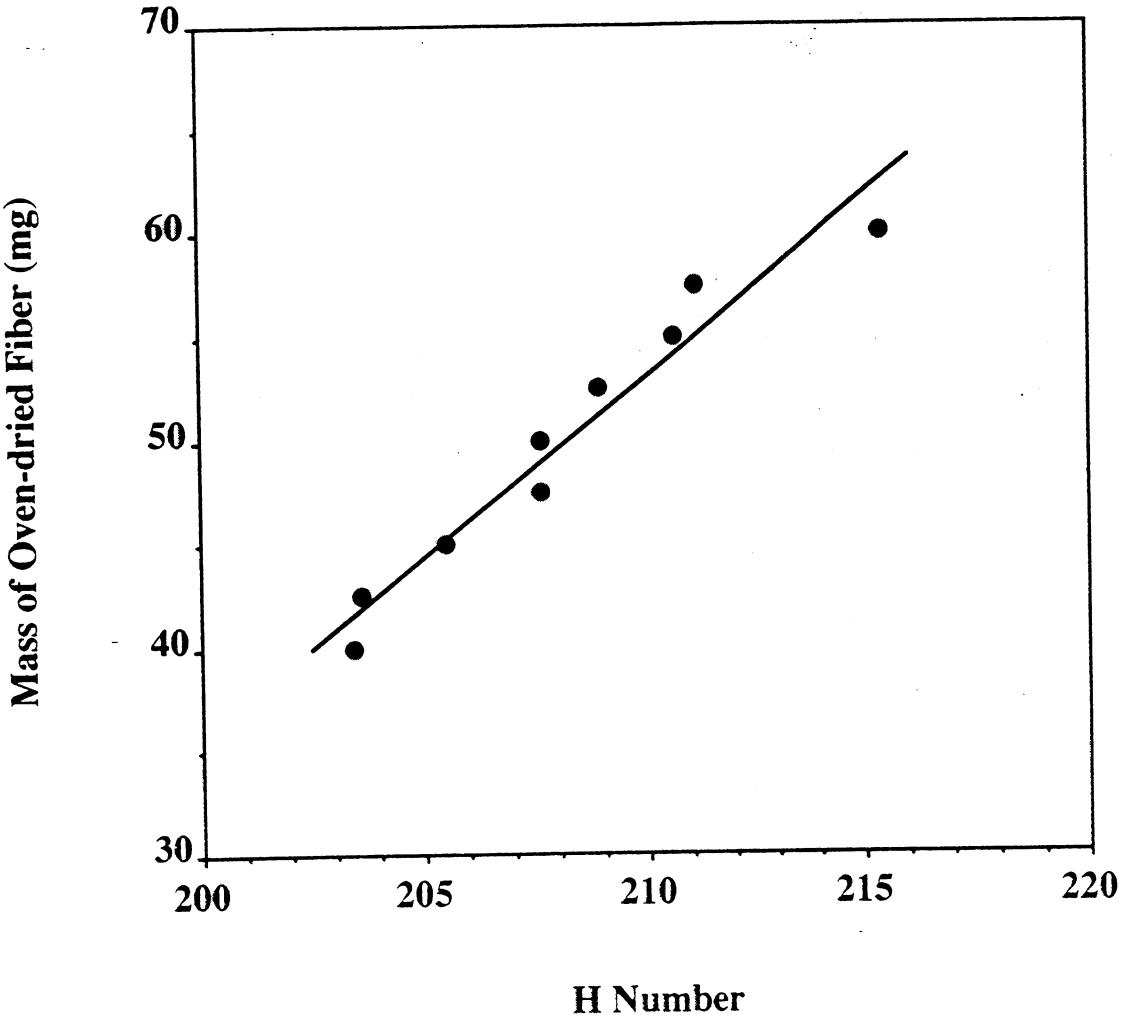
70
60
50
40
30
200
205
210
215
220
Mass of Oven-dried Fiber (mg)
H Number

Fig. 3

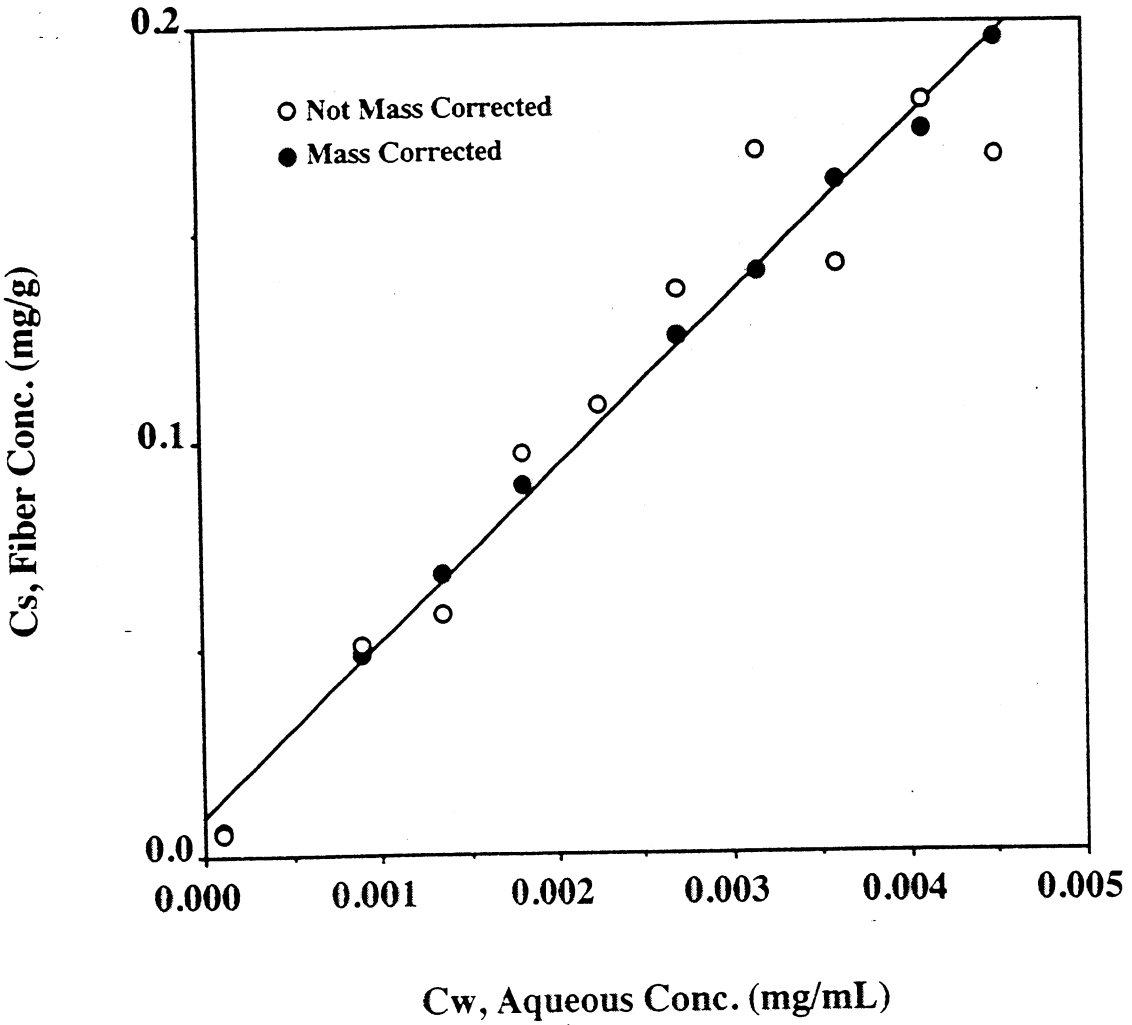
0.2
0.1
0.0
Not Mass Corrected
Mass Corrected
Cs, Fiber Conc. (mg/g)
0.000
0.001
0.002
0.003
0.004
0.005
Cw, Aqueous Conc. (mg/mL)

Fig. 4